



# Antibody-mediated autoimmunity in symptom-based disorders: position statement and proceedings from an international workshop

Rebecca Mountford<sup>a,\*</sup>, Brittany L. Adler<sup>b</sup>, David Andersson<sup>c</sup>, Rachael Bashford-Rogers<sup>d</sup>, Richard Berwick<sup>a,c</sup>, Stuart Bevan<sup>c</sup>, Xavier Caro<sup>e</sup>, Tae Hwan Chung<sup>f,g</sup>, J. David Clark<sup>h</sup>, John M. Dawes<sup>i</sup>, Xinzhong Dong<sup>j</sup>, Zsuzsanna Helyes<sup>k,l,m</sup>, Wade Kingery<sup>n</sup>, Joost J. van Middendorp<sup>o</sup>, Harvey Neiland<sup>a</sup>, Margot Maurer<sup>c</sup>, Carmen Scheibenbogen<sup>p</sup>, Katharina Schmack<sup>q,r</sup>, Thomas Schreiner<sup>s</sup>, Camilla I. Svensson<sup>t</sup>, Valéria Tékus<sup>k,u</sup>, Andreas Goebel<sup>a,v</sup>

## Abstract

A 2-day closed workshop was held in Liverpool, United Kingdom, to discuss the results of research concerning symptom-based disorders (SBDs) caused by autoantibodies, share technical knowledge, and consider future plans. Twenty-two speakers and 14 additional participants attended. This workshop set out to consolidate knowledge about the contribution of autoantibodies to SBDs. Persuasive evidence for a causative role of autoantibodies in disease often derives from experimental “passive transfer” approaches, as first established in neurological research. Here, serum immunoglobulin (IgM or IgG) is purified from donated blood and transferred to rodents, either systemically or intrathecally. Rodents are then assessed for the expression of phenotypes resembling the human condition; successful phenotype transfer is considered supportive of or proof for autoimmune pathology. Workshop participants discussed passive transfer models and wider evidence for autoantibody contribution to a range of SBDs. Clinical trials testing autoantibody reduction were presented. Cornerstones of both experimental approaches and clinical trial parameters in this field were distilled and presented in this article. Mounting evidence suggests that immunoglobulin transfer from patient donors often induces the respective SBD phenotype in rodents. Understanding antibody binding epitopes and downstream mechanisms will require substantial research efforts, but treatments to reduce antibody titres can already now be evaluated.

## 1. Introduction

A 2-day closed workshop was held in Liverpool, United Kingdom, to discuss the results of research concerning the autoantibody causation of symptom-based disorders (SBDs), share technical

knowledge, and consider future plans. Twenty-two speakers and 14 additional participants attended.

Symptom-based disorders can be defined as conditions characterised predominantly by somatic and/or psychiatric

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

<sup>a</sup> Pain Research Institute, University of Liverpool, Liverpool, United Kingdom, <sup>b</sup>Division of Rheumatology, Johns Hopkins University, Baltimore, MD, USA, <sup>c</sup>Wolfson SPaRC, Institute of Psychiatry, Psychology & Neuroscience, King’s College London, London, United Kingdom, <sup>d</sup>Department of Biochemistry, University of Oxford, Oxford, United Kingdom, <sup>e</sup>Southern California Fibromyalgia Research & Treatment Centre, Northridge Hospital Medical Center Professional Building, Los Angeles, CA, USA, <sup>f</sup>Department of Physical Medicine and Rehabilitation, The Johns Hopkins Medical Institutions, Baltimore, MD, USA, <sup>g</sup>Department of Neurology, The Johns Hopkins Medical Institutions, Baltimore, MD, USA, <sup>h</sup>Department of Anesthesia, Stanford University School of Medicine, Redwood City, CA, USA, <sup>i</sup>Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom, <sup>j</sup>Solomon H. Snyder Department of Neuroscience, John Hopkins University School of Medicine, Baltimore, MD, USA, <sup>k</sup>Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary, <sup>l</sup>HUNREN-PTE Chronic Pain Research Group, University of Pécs, Pécs, Hungary, <sup>m</sup>PharmInVivo Ltd., Pécs, Hungary, <sup>n</sup>Palo Alto Veterans Institute for Research, Palo Alto, CA, USA, <sup>o</sup>Argenx, Ghent, Belgium, <sup>p</sup>Institute of Medical Immunology, Charité—Universitätsmedizin Berlin, Berlin Institute of Health, Berlin, Germany, <sup>q</sup>Francis Crick Institute, London, United Kingdom, <sup>r</sup>Division of Psychiatry, University College London, London, United Kingdom, <sup>s</sup>Miltenyi Biotec, Bergisch Gladbach, Germany, <sup>t</sup>Department of Physiology and Pharmacology, Centre for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden, <sup>u</sup>Department of Laboratory Diagnostics, University of Pécs, Pécs, Hungary, <sup>v</sup>Walton Centre NHS Foundation Trust, Liverpool, United Kingdom

\*Corresponding author. Address: Pain Research Institute, University of Liverpool, Liverpool, L9 7AL United Kingdom. Tel.: 0151 529 5835. E-mail address: r.mountford@liverpool.ac.uk (R. Mountford).

Copyright © 2024 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of The International Association for the Study of Pain. This is an open access article distributed under the Creative Commons Attribution-NoDerivatives License 4.0 (CC BY-ND) which allows for redistribution, commercial and non-commercial, as long as it is passed along unchanged and in whole, with credit to the author.

PR9 9 (2024) e1167

<http://dx.doi.org/10.1097/PR9.0000000000001167>

symptoms, rather than objectively identifiable signs.<sup>19</sup> These disorders are very common and are, jointly, responsible for far more life years lived with disability than disorders where signs are more prominent. Awareness about their relevance has increased on the background of the declining impact of fatal disorders in most countries.<sup>12</sup> Symptom-based disorders pose a high burden on individuals, families, and the economy, including through reduced ability to care for others, and reduced work participation. These disorders are typically not well understood, and there are few effective treatments.

Symptom-based disorders include chronic “unexplained” pains such as musculoskeletal low back pain, fibromyalgia syndrome (FMS), chronic migraine, complex regional pain syndrome, fatigue conditions, psychiatric conditions, unexplained itch, nausea, postural orthostatic tachycardia syndrome (POTS), “functional” gastrointestinal disorders, and others. Symptom-based disorders typically arise in overlapping phenotypes, including both somatic and psychiatric, and hence bridging the traditional divide between somatic and mental disorders.

Although psychological concepts have long been used to explain SBDs, the fact that these disorders have important biological contributions has recently been confirmed in the course of the recent COVID-19 pandemic, where many patients developed multiple SBDs after resolution of their acute infection with COVID-19.<sup>35</sup> Increasing evidence indicates that, in addition to infection, *triggers* for SBDs can include toxicity such as after fluoroquinolone use<sup>9</sup> and the experience of psychological trauma and distress.<sup>49</sup>

This workshop set out to consolidate knowledge about the contribution of autoantibodies to SBDs. Persuasive evidence for a causative role of autoantibodies in disease often derives from experimental “*passive transfer*” approaches, as first established in neurological research.<sup>42</sup> Here, serum immunoglobulin (IgM or IgG) is purified from donated blood and transferred to rodents, either systemically or intrathecally. Rodents are then assessed for the expression of phenotypes resembling the human condition; successful phenotype transfer is considered supportive of or proof for autoimmune pathology.<sup>37</sup> Alternative powerful methods that provide such proof include rodent immunisation with suspected autoantigen or demonstration of patient response to certain immune therapies.

Workshop participants discussed passive transfer models and wider evidence for autoantibody contribution to a range of SBDs. Clinical trials testing autoantibody reduction were presented (Appendix). Cornerstones of both experimental approaches and clinical trial parameters in this field were distilled and are listed below as a “Position Statement.” Short summaries of findings are presented in the subsequent “Proceedings” section.

## 2. Conclusion

Mounting evidence suggests that immunoglobulin transfer from patient donors often induces the respective SBD phenotype in rodents. Understanding antibody binding epitopes and downstream mechanisms will require substantial research efforts, but treatments to reduce antibody titres can already now be evaluated.

### Position statement

(1) Serum-IgG or IgM derived from patients with certain chronic pain disorders elicit corresponding phenotypes upon passive immunoglobulin transfer to rodents. Preliminary evidence

suggests that IgM may be the predominant pathogenic isotype early in pain disorders or after trauma, whereas proalgesic IgG is relevant for persisting pain conditions after one year.

- (2) The molecular binding targets of pertinent autoantibodies have not yet been identified. Preliminary evidence indicates binding to satellite glial cells in FMS.
- (3) Results from experiments conducted across SBDs indicate that the transferred antibodies affect rodent “behaviour,” including pain sensitivity, movement, and muscular grip strength but without causing overt tissue destruction or inflammation as is common to classical autoantibody-mediated disorders. Such classical autoimmune diseases include pemphigus, autoimmune thyroiditis, or neuromyelitis optica. We anticipate that this behavioural effect is afforded through binding-induced changes in cellular *function*.
- (4) Description of these antibodies as “*functional*” may however be confusing because the word “functional” in colloquial clinical discourse has assumed the meaning of not medically explained.<sup>24</sup> The term *function-modifying antibodies* is suggested.
- (5) Autoantibodies in SBD might also cause minimal structural changes. Recent investigations have, eg, demonstrated changes in epidermal nerve fibre density and/or nerve conduction abnormalities through microscopy and electromyography, respectively, in FMS.<sup>10,41</sup> The relevance of these findings for the FMS phenotype are currently unknown but are subject to ongoing investigations.
- (6) Quantity matters. The success of serum-antibody transfer generally depends on the quantity of transferred human immunoglobulin of any one type (IgG, IgM), with higher quantities eliciting stronger effects and quantities below certain thresholds having no effect. There are marked differences concerning minimal effective quantities between different models.
- (7) Physiological concentrations of human IgG antibodies in mouse plasma requiring transfer of approximately 8 mg/day for a 22 g mouse—similar to the original paradigm developed for myasthenia gravis<sup>42</sup>—are sufficient to elicit significant effects in FMS and complex regional pain syndrome (CRPS) models. Halving the dose in these models to 4 mg/kg substantially reduces phenotype expression.
- (8) For IgG transfer, initial data indicate that transfer of clinical *subphenotypes* is sometimes possible: IgG transfer from patients with lower CRPS pain intensities elicits a weaker hyperalgesia phenotype<sup>8</sup>; transfer of serum from patients with FMS who experience increased pain in ambient cold may selectively elicit increased cold sensitivity.<sup>18</sup> Testing IgG transfer from SBD subphenotypes may therefore shed further light on disorder mechanisms.
- (9) Central nervous system involvement can vary substantially between models; for example, enhanced CNS glia cell activation along pain pathways is evident in CRPS but not in FMS IgG transfer models.
- (10) The time course of rodent phenotype development also differs. Daily injection of 8 mg over 3 to 4 weeks is required for modelling CASPR 2 antibody-associated pain, whereas injection of 8 mg over 2 consecutive days elicits the FMS phenotype and a single dose of immunoglobulin from CRPS patients may cause sensitisation in the recipient animals in a fracture model. These differences presumably correspond to disparate downstream mechanisms, different antigen targets, variable antibody affinities, and/or other factors.
- (11) Choice of investigational method matters. Assessing phenomena in the rodents that most closely mirror patients’

symptoms appears appropriate. For example, in patients with CRPS or FMS, pain to blunt pressure is a much more prominent sensory abnormality than pain to light touch, distinguishing these conditions from most neuropathic pains.<sup>13</sup> Such blunt *pressure* pain can be assessed in rodents using the technically challenging Randall–Selitto method; by contrast, the more commonly used handheld von-Frey testing method is particularly suited to testing sensitivity to *touch*. Use of electronic von-Frey assessment is a hybrid method (pressure and touch). Direct comparisons between these assessment methods should establish the best testing approach.

- (12) Where there are no established tests, it may be possible to develop novel test types by closely studying patient report, and integrating mechanistic understanding, as recently described in the context of disc puncture–back pain<sup>21</sup> and hallucinations.<sup>38</sup> For example, the certainty of a false perception was recently linked to the length of time, which the mouse was prepared to wait for a reward after expression of an incorrect choice.
- (13) Some transfer models may require a second hit. We define “second hit” as an obligatory model element, in addition to immunoglobulin transfer, without which the phenotype is not reproduced in rodents. In 3 established models, second hits are essential:
- (i) Cast immobilisation in the postfracture IgM transfer model,<sup>20</sup>
  - (ii) Disc puncture in the back pain IgM transfer model, and
  - (iii) Hind paw incision in the IgG transfer model for persistent CRPS.
- By contrast, CASPR2 and FMS transfer models do not appear to require second hits. For clarity, in models (i) and (ii), the trauma triggers the *production of* proalgesic IgM antibodies in immunocompetent mice, causing pain hypersensitivities; B-cell-deficient mice lacking IgM production ability (and will be much less hypersensitive after suffering the respective trauma) will develop such hypersensitivities following passive transfer of IgM from either mice or humans who had been subjected to a *similar trauma*. By contrast, in model (iii), a very small trigger is employed, which by itself does not appear to cause long-lasting pain hypersensitivity and may not trigger the production of proalgesic IgM, so this model is used specifically for human-to-rodent passive transfer experiments.
- (14) Interestingly, initial evidence suggests that specific second hits (or tissue injuries) will produce specific proalgesic IgM, which is pathogenic only in the context of the very tissue injury in which it was raised: eg, IgM produced by mice exposed to the cast-fracture immobilisation model is obligatory to that model, causing hypersensitivities; when transferred to B-cell-deficient mice subjected to this model, this IgM will restore these same hypersensitivities in these B-cell-deficient mice; however, this same IgM is without effect when transferred to mice subjected to the disc puncture model.<sup>21</sup> These findings confirm that the nature of transferred proalgesic IgM autoantibodies, similar to the respective proalgesic IgG autoantibodies in FMS and CRPS models, differs between the conditions. Careful analysis of any trauma sustained by patients before the onset of their SBD may guide the future development of second hit models.
- (15) Patient selection is crucial when establishing new SBD transfer models. Pragmatic principles may aid development:
- (i) Selection of patient donors with severe SBD phenotypes,
  - (ii) Initial use of SBD patient donors who have concomitant non-SBD autoimmune conditions, provided that there is no association with relevant symptoms, and

(iii) Initial recruitment of patient donors with quickly developing SBD symptoms, eg, over weeks or months, These steps were considered independently by several workshop investigators in their model developments. For clarity, although such steps were also followed in FMS studies, it emerged that serum-IgG purified from patients with severe phenotypes were ubiquitously active.

- (16) In some instances, in established autoimmune conditions, autoantibodies cause harm to the fetus when they cross the placenta in the third trimester or to the newborn during breast feeding. The nature of induced damage or dysfunction can resemble the mother’s disorder such as in myasthenia gravis or may be distinct such as the anti-Ro-mediated conduction block in offspring of SLE mothers. Potential maternal vertical transmission of pathogenic effects of autoantibodies can be probed using immunoglobulin transfer to pregnant rodents.<sup>7,32</sup> Where clinical evidence in SBD supports potential links with disorders or symptoms in offspring, consideration of the use of such models is recommended.
- (17) Preliminary results suggest that the time course of clinical phenotype improvement in autoantibody-associated SBDs after plasma exchange may lag the reduction of antibody serum titres, presumably allowing downstream mechanisms to rebalance.<sup>17</sup> In clinical trials involving the reduction or removal of antibodies, as with the neonatal fragment crystallizable receptor (FcRn) receptor antagonists, or other drugs or plasma exchange, a 3-month trial duration may be insufficient, and a 6-month duration may be more informative.
- (18) Many SBDs are polysymptomatic. In response to clinical treatments expected to address the identified root autoimmune cause, the degree by which single-domain parameters change may differ between the patients. The sole use of one outcome domain may not sufficiently capture intervention effects. For example, in FMS trials, the use of function or quality of life outcomes may be equally or even more informative than using pain intensity as the sole primary outcome variable.

## Proceedings

### **Early passive transfer protocols, and results in complex regional pain syndrome and fibromyalgia syndrome—Andreas Goebel**

Andreas Goebel<sup>a,b</sup>

Email: andreasgoebel@rocketmail.com

<sup>a</sup>Pain Research Institute, University of Liverpool, Liverpool, United Kingdom; <sup>b</sup>Institute of Translational Medicine, University of Liverpool, Liverpool, United Kingdom

First plans for complex regional pain syndrome (CRPS) passive transfer experiments were developed in 2000 following recognition of patients’ abnormal serum test results in sensitive western blots for various infectious agents.<sup>15</sup> IgG transfer studies from donors with severe persistent CRPS resulted in reduced open field rearing<sup>16</sup> and rotarod abnormalities but without abnormal pain sensitivity.<sup>5</sup> Borderline increased rodent peripheral neuronal IgG staining was seen in 90 CRPS patients vs controls.<sup>45</sup> The addition of unilateral paw trauma (skin–muscle incision) from 2011 (Prof. Helyes laboratory, Liverpool patients), with IgG transfer from the day before trauma continuing for several days, reproduced in all tested CRPS preparations persistent mechanical hyperalgesia, transiently increased paw swelling, and increased paw-CGRP production, resembling important human

phenotypes. These changes were restricted to the injured paw.<sup>3</sup> Further experiments from 2015 confirmed abnormal excitability of c-fibres in skin–nerve preparations (Prof. Andersson laboratory, Liverpool patients), spinal cord/brain glia cell activation along nociceptive pathways, and abrogation of the phenotype by IL-1 antagonist treatment; injection mass and timing were demonstrated relevant.<sup>4,12</sup>

Following serendipitous observations in Liverpool in 2015, IgG transfer from patients with severe FMS was performed from 2017, which invariably re-produced hyperalgesias, c-fibre hypersensitivities (Prof. Andersson's laboratory, Liverpool and Swedish patients), and skin small fibre rarefaction, reduced night peak-time movement, with dedicated IgG binding to dorsal root ganglion cells and activation of satellite glia cells, but no binding to brain/spinal cord cells (Prof. Svensson's laboratory, Swedish patients). No trauma was required.<sup>7</sup>

The ubiquitous nature of these findings in CRPS and FMS was unexpected and may raise the question of similar mechanism in other SBDs.

### **Disc puncture autoimmunity model for back pain—Wade Kingery**

Wade Kingery<sup>a</sup>

Email: wkingery@stanford.edu

<sup>a</sup>*Palo Alto Veterans Institute for Research, California, USA*

Previously, we observed that B cells and autoantibodies mediated chronic nociceptive sensitization in the mouse tibia fracture model of complex regional pain syndrome (CRPS) and that CRPS patient antibodies were pronociceptive in muMT fracture mice. This study used a lumbar spinal disc puncture (DP) model of low back pain in wild-type (WT) and muMT mice (lacking mature B cells and antibodies) to evaluate pronociceptive adaptive immune responses. Wild-type disc puncture mice developed 24 weeks of hindpaw mechanical allodynia and hyperalgesia, grip weakness, and a conditioned place preference response indicative of spontaneous pain, but pain responses were attenuated or absent in muMT DP mice. Spinal cord expression of inflammatory cytokines, immune cell markers, and complement components were increased in WT DP mice and in muMT DP mice. Dorsal horn immunostaining in WT DP mice demonstrated glial activation and increased neuronal C5a receptor expression. Serum collected from WT DP mice and injected into muMT DP mice caused nociceptive sensitization, as did intrathecal injection of IgM collected from WT DP mice, and IgM immune complexes were observed in lumbar spinal discs and cord of WT DP mice. Serum from WT tibia fracture mice (see D. Clark's abstract) was not pronociceptive in muMT DP mice and vice versa, evidence that each type of tissue trauma chronically generates its own unique antibodies and targeted antigens. When chronic low back pain patient IgM antibodies were injected intrathecally into muMT DP mice, they had pronociceptive effects. These data further support the pronociceptive autoimmunity hypothesis for the transition from tissue injury to chronic musculoskeletal pain state.

### **Assessing the pathogenicity of autoantibodies from patients with chronic pain conditions using live sensory neurons—John Dawes**

John M Dawes<sup>a</sup>

Email: john.dawes@ndcn.ox.ac.uk

<sup>a</sup>*Nuffield Dept. of Clinical Neurosciences, University of Oxford, Oxford, United Kingdom*

Previous work from my laboratory has shown that autoantibodies are a mechanism to cause pain without inducing inflammation or tissue damage. For example, autoantibodies directed against CASPR2 (a component of the voltage-gated potassium channel complex) from neuropathic pain patients bind primary sensory neurons and disrupt ion channel function to increase neuron excitability and cause pain. Through the use of treatments such as Intravenous Immunoglobulin (IVIg) and plasma exchange (which block or remove circulating antibodies, respectively), clinical data suggests a role for autoantibodies in a number of different pain conditions. The use of passive transfer models in mice and the recapitulation of patient symptoms is critical in confirming pathogenicity. However, the development of a higher throughput assay to identify putative pathogenic antibodies would allow for the screening of many patient samples and streamline selection for in vivo testing. A key first step in the pathogenicity of CASPR2-Abs, as well as other pathogenic autoantibodies from neuropathic pain patients, is their binding to sensory neurons. Using mouse primary sensory neurons and human IPSC-derived sensory neurons, we have tested the binding of patient IgG using sera from a range of well-characterised pain patients, including fibromyalgia, complex regional pain syndrome, diabetic neuropathy, small fibre neuropathy, and sciatica and compared with healthy control samples. Initial studies found high levels of healthy control IgG binding to mouse sensory neurons. However, there was a higher percentage of binding from pain patients when analysis focussed on those samples with the strongest binding profiles. The same samples were also applied to human IPSC-derived sensory neurons, with higher levels of binding in pain cohorts than in healthy control. Using patient CASPR2-Abs as an example, we have now developed assays to assess antibody internalisation and the impact of antibodies on neuron viability and excitability. These approaches form a pipeline of assays used to narrow in on pathogenic autoantibodies so that their impact on pain sensitivity can be further studied and ultimately antigenic targets identified.

### **Autoantibody contributions to the tibia-fracture/immobilization model of complex regional pain syndrome—David Clark**

David J Clark<sup>a</sup>

Email: djclark@stanford.edu

<sup>a</sup>*Department of Anesthesia, Stanford University School of Medicine, California, USA*

The adaptive system of immunity is becoming recognized as a contributor to both common and enigmatic chronic pain states. Autoantibodies generated in the setting of complex regional pain syndrome (CRPS) represents one of the strongest examples of this type of immune contribution. Accumulating evidence hinted at autoimmune/autoantibody contributions, including gene association studies, the presence of large numbers of antigen-presenting cells in affected limbs, and reports of the efficacy of immunosuppression and plasmapheresis in some CRPS sufferers. Motivated by this work, passive transfer experiments involving plasma or purified Ig fractions were undertaken. Working independently, one team found that IgG isolated from patients with chronic CRPS strongly elongate the time course of nociceptive sensitization in a rodent model of limb injury (plantar incision). Electrophysiological studies suggest that the antibodies interact directly with sensory fibers to cause the observed sensitization. The cytokine IL-1beta may be essential for this sensitization. Other work has involved the passive transfer of serum and purified IgM and IgG to B-cell-deficient mice subjected

to tibia fracture and limb immobilization, a widely accepted model of CRPS. Using these methods, it was demonstrated that B cells are required for the CRPS-related phenotype to be manifest. The knockdown of B cells using rituximab was similarly effective in eliminating the established CRPS phenotype in CRPS fracture/cast model mice. Translational studies suggest that for model animals and patients with early (<1 year) CRPS, it is the IgM fraction that carries most of the pronociceptive impact. Target antigen discovery studies have identified several proteins that interact with CRPS-related autoantibodies, including keratin, cytostructural proteins, histones, and excitatory amino acid receptors. The intraplantar or intrathecal injection of purified IgM caused sensitization similar to systemic administration of CRPS plasma. Turning to the mechanisms involved, it was observed using C5a receptor–knockout mice and using the C5a receptor antagonist PMX-53 that activation of the complement cascade is required for human or rodent IgM to cause sensitization in the translational fracture/cast model. Finally, the administration of rodent or human CRPS immunoglobulin appears to activate microglia in the spinal cord and macrophages in skin that in turn produce cytokines, including IL-6, IL-1beta, and TNFalpha, that in turn support nociception. These results suggest that measures that limit B-cell-dependent autoantibody formation, support Ig elimination, inhibit complement system activation, or block cytokine signalling may be effective in CRPS.

These and other experiments suggest that redundancy may make treatment challenging.

- (1) Multiple neuropeptides (SP, CGRP) are upregulated in skin and peripheral nerve of the fracture limb and in corresponding spinal cord neurons and both of these neuropeptides can induce chronic pain and pronociceptive innate and adaptive immune responses after fracture.
- (2) Both sensory and sympathetic signalling can induce pronociceptive innate and adaptive immune responses after fracture.
- (3) Components of both the innate (keratinocytes, mast cells, macrophages, and microglia) and adaptive (germinal center B cells, Tfh cells, antibodies) immune systems can either directly express and secrete or stimulate other immune cells to express and secrete pronociceptive inflammatory mediators (IL-1, IL-6, TNF, NGF).
- (4) Multiple pronociceptive inflammatory mediators (cytokines: IL-1, IL-6, TNF, growth factor: NGF, and complement fragments: C3a, C5a, membrane attack complex) are chronically produced by immune cells after fracture.

### **Hallucinating mice—assessing the role of autoantibodies in psychosis—Katharina Schmack (contribution from Andreas Goebel)**

Katharina Schmack<sup>a,b</sup>

Email: katharina.schmack@crick.ac.uk

<sup>a</sup>Francis Crick Institute, London, United Kingdom; <sup>b</sup>Division of Psychiatry, University College London, London, United Kingdom

**Background:** The lifetime prevalence of hallucinations not related to either disease or drug intoxication in the general population is 5%. Hallucinations can be associated with chronic pain and often commence either before or soon after chronic pain onset.<sup>34,39</sup> The cause of such hallucinations is unknown, hampering treatment. A rodent model allowing investigation of their mechanistic basis would be welcome.

**Methods:** We operationalized hallucinations as false perceptions expressed with high certainty and (1) quantified such

hallucination-like perceptions (HALIP) in mice and (2) investigated their neuronal substrates.<sup>38</sup>

**Results:** Mice occasionally exhibit HALIPs with high certainty. The HALIP frequency in mice was increased after the administration of ketamine, a drug known to induce psychosis-like experiences in humans. Hallucination-like perceptions in mice were associated with activation of mesostriatal dopamine-dependent circuits and could be abrogated by the administration of the antipsychotic drug haloperidol.

**Conclusions:** Hallucinations can be modelled in rodents, and this model could be further interrogated following integration of passive transfer approaches. The frequent coincidence of hallucinations with chronic pain might allow for “directed serendipity” in identifying autoantibody-contributed hallucinations by selecting sera from such patient donors who have already been positively screened for function-modifying autoantibodies causing their corresponding pain condition.

### **Optimization of the complex regional pain syndrome passive transfer-trauma mouse model and metabolomic analysis of the plasma samples—Valéria Tékus and Zsuzsanna Helyes**

Valéria Tékus<sup>a,b</sup>, Zsuzsanna Helyes<sup>a,c,d</sup>

Email: valeria.tekus@aok.pte.hu, helyes.zsuzsanna@pte.hu

<sup>a</sup>Department of Pharmacology and Pharmacotherapy, Medial School, University of Pécs, Pécs, Hungary; <sup>b</sup>Department of Laboratory Diagnostics, University of Pécs, Pécs, Hungary; <sup>c</sup>HUNREN-PTE Chronic Pain Research Group, University of Pécs, Pécs, Hungary; <sup>d</sup>PharmInVivo Ltd., Pécs, Hungary

Since the therapy of CRPS is unsatisfactory, there is a great need to understand its pathophysiological mechanisms to identify novel drug targets. The relatively short duration (14 days) was a limitation of our previously established passive transfer-trauma translational mouse model; therefore, we aimed to prolong up to 45 days and analyze the mouse plasma metabolomic profile to determine key mediators and pathways.

Plantar skin–muscle incision mimicked microinjury, and purified plasma IgG of CRPS patient was injected i.p. every 3 or 4 days during a long experiment or daily throughout 7- or 14-day periods (metabolomic analysis). The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry, and hyperalgesia was calculated in comparison with the baseline values. Repeated CRPS IgG injection significantly increased incision-induced mechanical hyperalgesia by 40% to 50% as compared with IgG of healthy volunteers up to 37 days. Significantly increased hyperalgesia was detected also in the intact side from day 29.

Plasma metabolites were quantified by mass spectrometry (Biocrates MxP Quant 500) and evaluated by bioinformatics. Nineteen, mainly lipid mediators including sphingomyelins (SM C18:0; SM C18:1), diglycerides (DG16:1, DG18:0), and phospholipids (PCaa C38:5), as well as amino acid derivatives elevated significantly in the plasma of CRPS IgG-treated mice compared with the healthy IgG-treated group at both time points. They are mainly involved in fat metabolism, neuronal signal transduction, and inflammation regulation.

**Support:** National Brain Research Program 3.0, OTKA K-138046; OTKA FK-146283; Hungarian Research Network (Chronic Pain Research Group), Pécs, Hungary; TKP2021-EGA-16, RRF-2.3.1-21-2022-00015; Pain Relief Foundation, Liverpool; RRF-2.3.1-21-2022-00011 Translational Neuroscience National Laboratory.

### **Dorsal root ganglion macrophages and satellite glia cell involvement in the fibromyalgia syndrome IgG passive transfer model—Camilla Svensson**

Camilla Svensson<sup>a</sup>

Email: camilla.svensson@ki.se

<sup>a</sup>Department of Physiology and Pharmacology, Centre for Molecular Medicine, Karolinska Institute, Stockholm, Sweden

Although it is well recognized that alterations in central pain processes are part of the pathology in fibromyalgia, accumulating evidence shows that fibromyalgia is also associated with changes in the peripheral nervous system and the immune system. We have established a link between the adaptive immune system and fibromyalgia symptoms by assessing behavioral and cellular changes in mice after transfer of antibodies (IgG) from fibromyalgia subjects. We found that injection of fibromyalgia patient IgG, but not IgG from healthy controls, to mice induces pain-like behaviors in form of increased sensitivity to cold stimulation and reduced locomotor activity. After transfer, fibromyalgia IgG accumulates in the dorsal root ganglion (DRG) where they bind to satellite glia cells (SGCs). In the experimental setting, the binding was associated with morphological and transcriptional changes coupled to altered SGCs activity. In addition, IgG from individuals with fibromyalgia show a higher binding intensity to SGCs in human DRG sections compared with IgG from healthy controls. These findings suggest that autoantibodies could be part of fibromyalgia pathology. We examined how frequently fibromyalgia patients have anti-SGC antibodies and how anti-SGC antibodies associate with disease severity. Elevated anti-SGC IgG titers were associated with higher levels of self-reported pain, higher fibromyalgia impact questionnaire scores, and increased pressure sensitivity but was not associated with fibromyalgia duration, pain duration, Becks depression inventory, BMI, or age. We clustered the fibromyalgia serum samples based on the severity of the disease and found that the severe fibromyalgia group had elevated anti-SGC IgG compared with the mild fibromyalgia and control groups. Furthermore, the severe fibromyalgia group had elevated IgG binding to SGCs in human DRG sections compared with the mild fibromyalgia and control groups.

The DRG is highly vascularized, likely as a consequence of the high metabolic demands of DRG sensory neurons. In contrast to the blood–brain barrier and the blood–nerve barrier, it is well documented that the blood vessels supplying the sensory ganglia are more permeable to circulating molecules than those supplying the axons. We found that DRG endothelial patterning is highly regulated to create a tight barrier in arteries and arterial capillaries, which tapers off toward venous capillaries and veins, providing the neuronal soma and interstitial cells underlying the capsule with a high exposure to circulating macromolecules such as IgG. In fact, a subset of DRG macrophages positive for CD163 localized around endothelial cells filled with caveolae displayed phagocytic ability aligned with peak endothelial permeability. Although the role of the CD163+ macrophages in the context of FM and IgG-induced hypersensitivity is not yet defined, it is possible that this network of macrophages has developed to limit enhanced permeability in the DRG or to serve as a controlled introduction of macromolecules into the parenchyma as a mean of cross vascular communication. Further studies are warranted to elucidate the role of CD163+ macrophages in FM-associated pain. In summary, our results suggest that autoantibodies underlie fibromyalgia pain in a subgroup of patients and open up

for the possibility that both satellite glial cells and macrophages in the DRG play a pivotal role in this process. Thus, advancing our knowledge on the role of the immune system in fibromyalgia is critical as it could provide a new path to personalized treatment options that target autoantibodies or autoantibody production for symptom relief.<sup>18,27,31</sup>

### **Antibody removal by apheresis in symptom-based disorders—Thomas Schreiner**

Thomas Schreiner<sup>a</sup>

Email: thomas.schreiner@miltenyi.com

<sup>a</sup>Miltenyi Biotec, Germany

Apheresis is a method for the fast and efficient removal of antibodies from the blood of patients. The available technologies range from therapeutic plasma exchange over selective methods to highly specific immunoadsorption by affinity chromatography. The TheraSorb apheresis platform uses Sepharose-based columns as a matrix and recombinant antibody fragments derived from lama. The ligands recognize the constant region of the immunoglobulins' light chains. IgG (subclasses 1–4), IgM, IgA, IgE, and immune complexes are bound equally well. To increase the binding capacity, the system uses a pair of absorbers with repeating cycles of plasma loading and adsorber regeneration. In clinical routine, more than 90% removal of IgG within 3 treatment days is usually achieved. After elimination of antibodies from the vascular system by immunoadsorption, a rebound of antibodies occurs from other compartments of the body into the bloodstream. Repeated treatments lead to a zigzag curve of the immunoglobulin levels in the blood of the patient. Usually, 5 to 7 treatments are required to achieve a sufficient clinical effect. The technology is used for numerous autoantibody-mediated indications and in solid organ transplantation for the prevention of antibody-mediated rejection. In long-COVID or ME/CFS, several autoantibodies have been detected either by functional assays or by ELISA. Unpublished data of the manufacturer demonstrate that immune adsorption for patients with long COVID leads to clinical benefit in more than half of the people affected (n = 18). To confirm the preliminary data, several clinical trials are ongoing, including trials with randomization and sham apheresis controls. Other clinical trials focus on acute flare of multiple sclerosis, CIDP, and myasthenia gravis. Immunoadsorption is a safe method with a very low rate of adverse events but with proven clinical benefit in more than 50 different indications.

### **Passive transfer of chronic primary pain syndromes—David Andersson**

Margot Maurer<sup>a</sup>, Clive Gentry<sup>a</sup>, Nisha Vastani<sup>a</sup>, Mathilde Israel<sup>a</sup>, Ülkü Cuhadar<sup>a</sup>, Haoyue Sun<sup>a</sup>, Harvey Neiland<sup>b</sup>, Serena Sensi<sup>b</sup>, Uazman Alam, Anne Marshall, Andrew Marshall, Stuart Bevan<sup>a</sup>, Andreas Goebel<sup>b</sup>, David Andersson<sup>a</sup>

Email: david.andersson@kcl.ac.uk

<sup>a</sup>Wolfson SPaRC, Institute for Psychiatry, Psychology, and Neuroscience, King's College London, SE1 1UL; <sup>b</sup>Pain Research Institute, University of Liverpool, Liverpool, United Kingdom

**Background:** We have previously demonstrated that IgG from complex regional pain syndrome (CRPS) patients produced persistent, painful hypersensitivities and nociceptor sensitization in mice<sup>8</sup> treated with the established combination of IgG and a minor insult to transfer symptoms from CRPS patients to mice.<sup>23</sup> CRPS IgG required an insult to transfer symptoms, and hypersensitivities were limited to the injured

paw. These results prompted us to consider whether other chronic pain conditions share an autoantibody-mediated pathophysiology with CRPS.

We next examined whether fibromyalgia (FMS) IgG could transfer symptoms to mice.<sup>1</sup> Using behavioural, electrophysiological, and imaging methods, our studies demonstrated that administration of patient IgG transferred sensory and motor symptoms and anatomical signs of FMS to mice.<sup>18</sup>

**Methods and Results:** Since paraesthesia is common in FMS, we explored the sensory qualities of cold evoked sensory abnormalities in patients. Intriguingly, patients are less likely to describe cold pain as “cold” but often as “pins and needles” and “tingling,” descriptors commonly associated with paraesthesia and A $\beta$ -fibre dysfunction. Electrophysiological investigations confirmed that FMS IgG produced functional abnormalities, including an aberrant cold responsiveness, in A $\beta$ -fibres in mice. Transcriptomic investigations identified downregulation of a leak K<sup>+</sup>-channel (KCNK3) as the sole dysregulated ion channel in DRGs from mice treated with FMS IgG. Inhibition of this channel recapitulated hypersensitivities produced by FMS IgG in vivo and in vitro.

**Discussion:** Based on our work,<sup>18</sup> therapeutic IgG reduction by the inhibition of FcRn-mediated recycling of IgG is being evaluated in FMS. Experimentally, we are currently exploring the potential of pharmacological interventions targeting KCNK3.

**Support:** Funding from the Sir Jules Thorn Award for Biomedical Research (22/JTA, the MRC (MR/S003428/1, MR/W002426/1, MR/W027585/1) and Versus Arthritis (21544).

### ***In vivo imaging in the fibromyalgia syndrome passive transfer model—Xinzhong Dong***

Qin Zheng<sup>a</sup>, Karla Sanchez<sup>a</sup>, Xinzhong Dong<sup>a</sup>  
Email: xdong2@jhmi.edu

<sup>a</sup>*Solomon H. Snyder Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, USA*

Pain-sensing neurons in dorsal root ganglion (DRG) play an essential role in pain transmission and modulation. Although the cell bodies reside in the DRG, the axons of these primary sensory neurons innervate peripheral tissues where they detect painful stimuli. Pain signals are transmitted from the periphery to their central axonal terminals in the spinal cord's dorsal horn, where they synapse onto secondary neurons. It is recognized that tissue injury induces hyperexcitability or sensitization of primary afferent neurons, a major contributor to chronic pain. By specifically expressing a genetically encoded Ca<sup>2+</sup> sensitive indicator in almost all DRG neurons in Pirt-GCaMP6 mice, we successfully imaged lumbar DRG at populational level in live mice. Using this technique, which allows us to monitor the activation of >1,600 neurons at the same time, we have revealed several novel mechanisms under chronic pain conditions, such as neuronal coupling and synchronized cluster fire. In addition, we have used Pirt-GCaMP6 mice with in vivo DRG imaging in the FMS IgG passive transfer model. We found that injection of IgG from FMS patient evoked robust neuronal hypersensitivity in the DRG in response to mechanical (brushing and pinching) and cold stimuli as compared with IgG from healthy controls. Consistently, FMS IgG induced robust mechanical allodynia and cold hyperalgesia, whereas healthy IgG did not. Together, these data suggest that the FMS passive transfer model causes pain in mice, which is resulted from the elevated DRG neuronal activation. The mechanism underlying the neuronal hypersensitivity requires further investigation.

### ***Investigations on satellite glial cells and innate immune cells in fibromyalgia syndrome, complex regional pain syndrome and post-COVID syndrome—Richard Berwick and Harvey Neiland***

Richard Berwick<sup>a,b</sup>, Harvey Neiland<sup>a</sup>

Email: richard.berwick@liverpool.ac.uk, hlnheila@liverpool.ac.uk  
<sup>a</sup>*Pain Research Institute, University of Liverpool, Liverpool, United Kingdom;* <sup>b</sup>*Wolfson SPaRC, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom*

**Background:** Fibromyalgia syndrome (FMS) and complex regional pain syndrome (CRPS) may have an autoantibody-mediated pathophysiology.<sup>18,21</sup> Swedish and Canadian patient IgG was found to be autoreactive to satellite glial cells (SGC). We set out to confirm this in a UK FMS cohort and explore SGC staining in CRPS and painful post-COVID syndrome (PCS).

**Methods:** Serum or plasma from FMS/CRPS diagnosed patients and pain-free controls were collected (Walton Centre, United Kingdom). Patients had high pain ( $\geq 7/10$ ). post-COVID syndrome patients were recruited at Charité Hospital, Germany<sup>20</sup> and fulfilled criteria for chronic fatigue syndrome, being previously well; their serum was group-pooled high pain (PC++)  $\geq 7/10$  n = 5, low pain (PC+)  $\sim 1$  to 4/10 n = 4, and recovered patients (RC) 0/10 n = 6. For ex vivo FMS staining, a female C57BL/6J rodent was injected intraperitoneally (8 mg protein-G affinity-purified IgG/day) for 3 days and harvested on day 4. Staining of mouse SGC-enriched cultures were compared between these injected and uninjected mice.<sup>18</sup> Cultures were incubated with 10  $\mu$ g/mL IgG (3 hours) and labelled for human IgG. Cells were imaged, and fluorescence intensity was measured.

**Results:** FMS SGC IgG staining was significantly enhanced compared with HC-IgG-treated cells. In vivo IgG injection before in vitro SGC IgG incubation demonstrated IgG-antigenicity downregulation in the FMS group. For CRPS, SCG-IgG staining was inconsistent. For PCS, SGC-IgG staining was greater when treated with PC++ IgG compared with RC-IgG. However, when individual samples were tested, this was not replicated.

UK FMS patients with high pain produce anti-SCG antibodies confirming earlier results by others. In CRPS, we find significant variation; sensitising trauma, as in the mouse model, may be required. The presence of autoantibodies in PCS is not uniform and pooled samples are skewed by high-staining individuals. We expect that PCS phenotyping is key because pain may be high but not widespread.

### ***What do we know about autoantibodies in postural orthostatic tachycardia syndrome—Brit Adler***

Brit Adler<sup>a</sup>

Email: brit.adler@jhmi.edu

<sup>a</sup>*Division of Rheumatology, Johns Hopkins University, Baltimore, USA*

Fibromyalgia and other chronic pain syndromes often overlap with other syndromes including myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and autonomic dysfunction/postural orthostatic tachycardia syndrome (POTS). Postural orthostatic tachycardia syndrome results from impaired sympathetic vasoconstriction leading to venous pooling and reduced cerebral perfusion in the upright position and a compensatory tachycardia. Both CFS and POTS are often triggered by an infectious insult and are important components of the long COVID syndrome.<sup>28</sup> A small-fiber neuropathy is present in a subgroup of patients with CFS and POTS,<sup>13</sup> suggesting

a shared pathogenesis with fibromyalgia, which is also associated with the presence of a small-fiber neuropathy.

A small subset of patients with dysautonomia have a proven autoimmune disease called autoimmune autonomic ganglionopathy (AAG) and have pathogenic autoantibodies targeting the ganglionic nicotinic acetylcholine receptor (AChR).<sup>46</sup> Patients with these autoantibodies have autonomic failure, including orthostatic hypotension, urinary retention, constipation, sicca syndrome, and dilated pupils, and have an excellent response to immunomodulation.<sup>25</sup> Passive transfer of ganglionic acetylcholine receptor antibodies reproduces autonomic symptoms in mice.<sup>47</sup> However, some patients with AAG do not have detectable AChR antibodies but still respond well to immunosuppression, suggesting that other autoantibodies remain to be discovered in some autonomic syndromes.<sup>25</sup> Patients with POTS frequently have immune dysregulation with an abnormal humoral antibody response. Patients with POTS are more likely to have a positive ANA and a higher prevalence of antithyroid antibodies and antiphospholipid antibodies.<sup>2</sup> Recent studies have demonstrated that the majority of patients with POTS and ME/CFS have autoantibodies targeting adrenergic and muscarinic G-protein-coupled receptors (GPCR), which are receptors that play a pivotal role in the autonomic nervous system.<sup>11,30</sup> Some studies have shown that these autoantibodies correlate with disease severity,<sup>40</sup> and serum from these patients activate GPCR receptors to a higher degree than that from controls.<sup>26</sup> However, the presence of GPCR autoantibodies in POTS is controversial and has not been reproduced by other groups.<sup>22</sup> Other autoantibodies targeting trisulfated heparin disaccharide (TSHDS) and fibroblast growth factor receptor 3 (FGFR3) have been identified in patients with idiopathic small-fiber neuropathy and dysautonomia.<sup>29,43</sup> However, it is unclear if these autoantibodies are pathogenic, and recent small studies have not shown that patients with these autoantibodies have a clinical response to immunomodulation.<sup>14</sup>

Larger therapeutic trials of immunomodulation for patients with POTS are ongoing, as well as studies to identify novel autoantibodies that can serve as biomarkers for dysautonomia and small-fiber neuropathy. Given the significant overlap between ME/CFS, POTS, fibromyalgia, and small-fiber neuropathy, it is possible that there may also be shared autoantigens between these syndromes. Passive transfer experiments are also needed to determine whether autoantibodies in POTS are pathogenic.

### **Autoantibodies in myalgic encephalomyelitis/chronic fatigue syndrome and the effect of immunoglobulin depletion by immunoadsorption—Carmen Scheibenbogen**

Carmen Scheibenbogen<sup>a</sup>

Email: carmen.scheibenbogen@charite.de

<sup>a</sup>*Institute of Medical Immunology, Charité—Universitätsmedizin Berlin, Berlin Institute of Health, Berlin, Germany*

**Background:** Many infectious diseases are associated with postinfectious sequelae. Myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) is one of the most disabling and incurable postinfectious syndromes with an estimated doubling during the pandemic, thus affecting at least 0.5% of the population. Autoimmunity is postulated to play a major role in the pathophysiological mechanisms of ME/CFS. The  $\beta$ 2-adrenergic receptor antibody (ADRB2 AAB) is found elevated in a subgroup of patients, and fatigue, neurocognitive impairment, vasomotor symptoms, and structural central nervous system alterations are associated with the levels of ADRB2 AABs in ME/

CFS patients. There is first evidence that treatments targeting AABs, including B-cell depletion and immunoadsorption, may have efficacy in ME/CFS.

**Methods/Results:** Results from small observational trials provide first evidence for efficacy of immunoadsorption in patients with postinfectious and post-COVID-19 ME/CFS. The results of these observational studies provide the basis for patient selection for a randomised controlled trial (RCT), including sham apheresis, and for a RCT combining immunoadsorption with B-cell-depletion therapy.

### **Neuronal autoantibodies in post-COVID syndrome—Stuart Bevan**

Hayate Javed<sup>a</sup>, Helen E. Davies<sup>b</sup>, Samantha A. Jones<sup>b</sup>, Kelly L. Miners<sup>c</sup>, Kristin Ladell<sup>c</sup>, David A. Price<sup>c</sup>, David A. Andersson<sup>a</sup>, Stuart Bevan<sup>a</sup>

Email: stuart.bevan@kcl.ac.uk

<sup>a</sup>*Wolfson SPaRC, Institute for Psychiatry, Psychology, and Neuroscience, King's College London, London, United Kingdom;* <sup>b</sup>*Department of Respiratory Medicine, University Hospital of Wales, Llandough, Penarth, United Kingdom;* <sup>c</sup>*Division of Infection and Immunity, Cardiff University School of Medicine, University Hospital of Wales, Heath Park, Cardiff, United Kingdom*

Post-COVID syndrome (PCS), also known as long-COVID, has a prevalence of 10% to 20% in individuals previously infected with SARS-CoV-2. In the United Kingdom, 70% and 40% of patients reporting PCS have symptoms that have persisted for >1 and >2 years, respectively. The most common symptoms of PCS include fatigue, pain, increased sensitivities, and dysautonomia. These symptoms are strikingly similar to those seen in patients with fibromyalgia syndrome (FMS). Recent studies have revealed that the sensory and motor symptoms of patients with FMS can be passively transferred to mice by the administration of IgG antibodies from patients with FMS.<sup>18</sup> This finding is consistent with an autoimmune aetiology for FMS. After passive transfer to mice, IgG from patients with FMS is seen in association with satellite glial cells (SGCs) in sensory ganglia and functionally sensitizes sensory nerves.

Autoantibody production has been reported in patients infected with SARS-CoV-2,<sup>48</sup> which, together with the noted symptom overlap between FMS and PCS, raises the possibility that some symptoms in patients with PCS may be autoantibody mediated. Therefore, we are studying the effects of PCS IgG on sensory function in mice. Immunostaining studies revealed that IgG from PCS patients with high pain scores showed greater binding to mouse sensory neurons than IgG from post-SARS-CoV-2-infected individuals with no pain. By contrast, IgG from PCS patients with pain or post-SARS-CoV-2-infected individuals without pain showed similarly weak binding levels to mouse SGCs. Ongoing passive transfer studies will determine the effects of PCS IgG on neuronal function and sensitivities in mice.

**Support:** Research supported by MRC & Versus Arthritis grant MR/W027623/1.

### **The potential role of dysfunctional B-cell selection in fibromyalgia syndrome—Rachael Bashford-Rogers**

Rachael Bashford-Rogers<sup>a</sup>

Email: rachael.bashford-rogers@bioch.ox.ac.uk

<sup>a</sup>*Department of Biochemistry, University of Oxford, Oxford, United Kingdom*



**Background and Aims:** The recent discovery of the transfer of fibromyalgia symptoms (FMS) through pain-sensitive IgG has suggested the involvement of B cells in the development of FMS. Immunological health relies on a balance between the ability to mount an immune response against potential pathogens and tolerance to self. B cells are key players in the immune response through the generation of B-cell receptors or, when secreted, antibodies. Defects in tolerance mechanisms lead to auto-inflammatory diseases. Here, we combined both high-throughput BCR sequencing, a powerful tool for dissecting the BCR populations at high resolution, and a detailed comparison of differences in B-cell populations in FMS to bridge the gap in the understanding of the immunological perturbations in FMS and build an evidence base for further studies.

**Methods:** PBMCs were obtained from blood samples of 36 participants, of which 17 were diagnosed with FMS and 19 were healthy controls. The patient cohort mostly consisted of females; to match this, healthy controls had a similar sex distribution. All participants were Caucasian. Second, we used the data set from Verma et al. in which FACS was performed on the blood immune cells of 44 FM patients and 46 matched healthy controls, with a focus on NK cells. Analysis of the B-cell population frequencies was performed on the BMD panel.

**Results:** We show that broad FMS BCR repertoire characteristics were distinct from healthy controls. Specific differences included reduced class-switching, elevated clonality in class-switched B cells, distinct somatic hypermutation (SHM) patterns, and distinct IGHV/J gene usages in antigen-experienced B cells in FMS patients. The FACS analysis revealed changes in subpopulations of B cells of FMS patients with some common characteristics with other autoimmune diseases, such as SLE and RA, including increased proportions of CD27-/IgD B cells and increased proportions of CD27+/IgD+/CD38-switched memory B cells.

These observations all point towards significant defects in peripheral tolerance, with minimal evidence of defects in central tolerance. In agreement with this, through the analysis of FACS data, we observed that some antigen-experienced B-cell population were present at differential frequencies in FMS patients compared with controls. The B-cell subset frequency differences share some commonalities with other autoimmune diseases, such as SLE and RA, including increased proportions of CD27-/IgD B cells and increased proportions of CD27+/IgD+/CD38-switched memory B cells.

**Conclusions:** Both the BCR sequencing and FACS analyses suggest differences in antigen-experienced B-cell selection, survival, and differentiation potential between FMS patients and controls. Together, this study provides key insights into distinct peripheral B-cell tolerance in FMS patients and potential B-cell dysfunction in FMS.

**Support:** We thank Andreas Goebel for project coconceptualisation, Richard Berwick for performing blood sample collection and PBMC isolation for the BCR sequencing study cohort, Antonio Choi Choi, Felicia Tucci, and Orthi Onupom for BCR library preparation, sequencing and FACS analysis, and Shihong Wu and Jane Pernes for troubleshooting. This was supported by the Department of Biochemistry (University of Oxford).

### **Role of the CX3CR1 fractalkine receptor in the complex regional pain syndrome passive transfer trauma model—Zsuzsanna Helyes and Valéria Tékus**

Zsuzsanna Helyes<sup>a,b,c</sup>, Valéria Tékus<sup>a,d</sup>

Email: helyes.zsuzsanna@pte.hu, valeria.tekus@aok.pte.hu

<sup>a</sup>Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary; <sup>b</sup>HUNREN-PTC Chronic Pain Research Group, University of Pécs, Pécs, Hungary; <sup>c</sup>PharmInVivo Ltd., Pécs, Hungary; <sup>d</sup>Department of Laboratory Diagnostics, University of Pécs, Pécs, Hungary

**Background and Aims:** Complex regional pain syndrome (CRPS) is a severe primary chronic pain condition accompanied by edema and autonomic disorders after a small injury. The pathophysiological mechanisms are unknown, but immune response against sensory nerve-derived antigens, complex neuroimmune–vascular interactions, and neuroinflammation are suggested to be involved. Because the treatment is unsatisfactory, identifying key mediators and novel therapeutic targets are necessary. The inflammatory chemokine fractalkine receptor 1 (CX3CR1) expressed on microglia cells and macrophages plays a role in neuroinflammatory processes. Here, we investigated its involvement in a passive transfer-trauma translational CRPS mouse model.

**Methods:** Female CX3CR1 deficient and wildtype mice were treated daily with plasma IgG purified from CRPS patients or healthy volunteers. Plantar skin–muscle incision was performed on day 0 to model the microinjury. The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry, astrocyte and microglia in pain-related central nervous system regions by glial fibrillary acidic protein (GFAP) and Iba1 immunohistochemistry. The CX3CR1 antagonist AZD 8797 (80 µg/kg i.p.) was administered daily to wild-type mice.

**Results:** Daily i.p. injections of CRPS IgG significantly increased mechanical hyperalgesia, as well as astrocyte and microglia markers in the spinal cord dorsal horn, periaqueductal gray, and somatosensory cortex during the 7-day experimental period after plantar skin–muscle incision, compared with healthy IgG treatment in wild-type animals. Both CX3CR1 deficiency and the antagonist treatment significantly diminished the CRPS IgG-induced increased pain behavior and glia cell activation.

**Conclusions:** CX3CR1 activation is likely to mediate CRPS-associated pain and neuroinflammatory mechanisms, suggesting that CX3CR1 inhibition might provide novel analgesic perspectives.

**Support:** National Brain Research Program 3.0; OTKA K-138046; Hungarian Research Network- Chronic Pain Research Group, Pécs, Hungary; TKP2021-EGA-16; RRF-2.3.1-21-2022-00011 Translational Neuroscience National Laboratory.

### **In utero passive transfer studies to assess autism spectrum disorder in children of patients with fibromyalgia syndrome—Andreas Goebel**

Rebecca Mountford<sup>a</sup>, Andreas Goebel<sup>a,b</sup>

Email: andreasgoebel@rocketmail.com

<sup>a</sup>Pain Research Institute, University of Liverpool, Liverpool, United Kingdom; <sup>b</sup>Walton Centre NHS Foundation Trust Liverpool, United Kingdom

**Background and Methods:** Clinical observation of report of autism spectrum disorder (ASD) in their children, of patients with severe FMS seen in clinical practice at the Walton Centre in Liverpool prompted retrospective review of all clinic letters of one consultant over 2.5 years (service evaluation).

**Results:** Of 590 screened new-patient outpatient clinic letters 90% indicated that patients had been asked about their children's health. Between 15% to 17% of all patients who indicated that they had biological children and who were diagnosed with severe FMS (n = 85) or severe CRPS (n = 37) reported that at least one child had autism (UK parent reported average > 2%).

The incidence in children of patients with other pains ( $n = 235$ ) was 5% (group difference significant,  $P > 0.02$ ).

**Discussion and Plans:** In established autoantibody-mediated autoimmune conditions, such as CASPR2-associated disease, ASD in biological offspring of mothers has been investigated as possible consequence of the transfer of neurodevelopmentally harmful IgG antibodies, and potential harm has been demonstrated in some cases.<sup>33</sup>

An ethics application to confirm our findings and to enquire about relevant parameters in more detail is being developed. In parallel, a passive-transfer study protocol to investigate any harmful neurodevelopmental effects is being compiled aiming to inject pregnant mice with patient IgG. Any findings would have important clinical implications, both for patient information, possible treatment during pregnancy with IgG-reducing interventions, and early diagnosis of any neurodevelopmental problems in children.

### **FcRn trials in post-COVID syndrome and postural orthostatic tachycardia syndrome—Tae Hwan Chung and Joost van Middendorp**

Tae Hwan Chung<sup>a,b</sup>, Joost van Middendorp<sup>c</sup>

Email: tchung7@jhmi.edu, jvanmiddendorp@argenx.com

<sup>a</sup>Department of Physical Medicine and Rehabilitation, The Johns Hopkins Medical Institutions, Baltimore, USA; <sup>b</sup>Department of Neurology, The Johns Hopkins Medical Institutions, Baltimore, USA; <sup>c</sup>Argenx, Belgium

**Background:** Although the underlying pathophysiology is unknown, post-COVID-19 postural orthostatic tachycardia syndrome (POTS) may be related to immune dysfunction and autoimmunity precipitated by SARS-CoV-2 infection. Various autoantibodies, including those targeting G-protein-coupled receptors (GPCRs), have been observed in patients with POTS. Binding of these autoantibodies to adrenergic receptors, which are prototypical GPCRs, has been hypothesized to modulate receptor activity, increase sympathetic tone, and cause tachycardia. Moreover, IgG anti-GPCR autoantibodies, acting as partial agonists, are thought to decrease the effect of peripheral vasomotor control, leading to an increased sympathetic response to upright posture that can result in postural tachycardia in the absence of hypotension.

**Objectives:** We hypothesize that a reduction in autoantibody levels by efgartigimod, a FcRn-antagonist, will ameliorate the underlying immune-mediated pathogenesis and lead to clinical improvements in patients who developed POTS following COVID-19 infection. We propose a phase II, multicenter, randomized, placebo-controlled, double-blind, proof-of-mechanism study initiated to evaluate the safety and efficacy of efgartigimod in adults with post-COVID-19 POTS.

**Methods:** Adult patients diagnosed with new onset of POTS following SARS-CoV-2 infection are eligible for participation in the study. Prior COVID-19 must be confirmed by documentation of historical PCR test, and POTS diagnosis must meet consensus criteria. Study subjects must have moderate to severe autonomic symptoms (COMPASS-31 score C 35 points at screening). Approximately 42 patients will be randomly allocated (2:1) to receive weekly intravenous efgartigimod or placebo for 24 weeks. At the end of the treatment period, participants who complete the study may roll over into an open-label extension (OLE). The coprimary endpoints are change from baseline to week 24 in the COMPASS-31 and Malmö POTS Symptom Score as well as safety and tolerability outcomes. Key secondary endpoints include assessments of disease activity, fatigue, cognitive

function, walking capacity, and quantitative autonomic testing. Additional exploratory endpoints include intraepidermal small fiber densities (optional), sudomotor innervation (optional), and peripheral blood biomarkers to define histopathological and immune determinants associated with treatment response.

**Conclusions:** This phase II study of efgartigimod will evaluate the effect of FcRn inhibition on disease pathology and the potential for therapeutic benefit in patients with post-COVID-19 POTS.

**Funding:** This study is sponsored by argenx.

### **Treatment of patients with fibromyalgia syndrome using IVIG and plasma exchange—Xavier Caro**

Xavier Caro<sup>a</sup>

Email: xjcaro@earthlink.net

<sup>a</sup>Southern California Fibromyalgia Research & Treatment Centre, Northridge Hospital Medical Center Professional Building, California, USA

Xavier J. Caro reviewed published data from as far back as 1984, showing that biopsies from clinically normal fibromyalgia (FM) skin demonstrate granular IgG deposition along the dermal-epidermal junction (DEJ) and within the dermal stroma.<sup>6</sup> This finding, he pointed out, has never been described as normal and likely signals “enhanced vascular permeability,” a microscopic and macroscopic phenomenon known to be associated with visceral inflammatory responses. He also pointed out the intriguing coincidental description of an anatomically congruent cutaneous dermal and epidermal nerve fiber injury pattern that has been described in FM from multiple centers.<sup>5,36,44</sup> This latter feature is commonly associated with small fiber neuropathy (SFN), a known peripherally painful condition. He ended his early remarks by pointing out that pathological afflictions have been identified in nearly all portions of the nervous system in FM, including the CNS, dorsal root ganglia, and small nerve fibers innervating the DEJ, and juxtaposed sweat glands. The ostensible sole exception to these diffuse nerve tissue lesions, large nerves in FM, were the subject of his next remarks. Clinical observation has shown, he pointed out, that FM patients complain of, and have findings of, significantly weaker proximal muscles than controls.<sup>4</sup> Furthermore, careful electromyographic and nerve conduction studies in FM patients have shown that approximately 90% have a demonstrable polyneuropathy with 40%–45% meeting electrodiagnostic criteria for designation as chronic inflammatory demyelinating polyneuropathy (CIDP).<sup>5</sup> These findings, he pointed out, are supported by ultrastructural morphometric analysis of FM sural nerve fascicles and teased single nerve fibers conducted at a university neuromuscular laboratory.<sup>5</sup> Taken together, these findings suggest that large nerve fiber pathology is often overlooked in FM, perhaps due to the misapplication of peripheral electrodiagnostic testing. This oversight precludes the use of modalities such as intravenous immune globulin and plasmapheresis in FM subjects who would benefit from such treatment.<sup>4</sup> Dr. Caro concluded by pointing out the value of viewing the FM patient in a holistic fashion, keeping in mind the diffuse—likely immune mediated—nature of the FM neuromuscular injury pattern, which allows for more complete and systematic understanding of FM complaints and their treatment.

### **A randomised, controlled trial of Rozanolixizumab in fibromyalgia syndrome—Andreas Goebel**

Andreas Goebel<sup>a</sup>

Email: andreasgoebel@rocketmail.com

<sup>a</sup>Pain Research Institute, University of Liverpool, Liverpool, United Kingdom

**Background:** Severe fibromyalgia syndrome (FMS) was recently demonstrated associated with proalgic IgG autoantibodies that can explain aspects of the clinical phenotype. FcRn receptor blockers are a new drug class that can reduce plasma IgG antibody concentration (all subclasses) by 65% to 75% through augmentation of intracellular protein breakdown.

**Methods:** The authors designed a proof-of-concept RCT to test the efficacy and safety of rozanolizumab in FMS, an FcRn drug which recently received FDA approval for the treatment of myasthenia gravis (an autoantibody-mediated disorder).

Taking into account results from plasma exchange treatment of 3 FMS patients at the Liverpool Walton Centre and from laboratory studies,<sup>18,27</sup> we (1) included patients with an average baseline-period pain intensity  $\geq$ NRS6 <10NRS, (2) selected functional improvement (BPI interference) as primary outcome measure, recognising that hypothetical quality of life improvements following serum-IgG reduction will result from distinct individual changes in eg, fatigue, pain, hyperalgesia, and coalescing in the common outcome of better function, (3) timed the primary outcome assessment after 3 months of treatment duration, with the ability to additionally test outcomes after an additional 3 months.

**Results:** Study recruitment was in NW England and aimed at the recruitment of 60 patients.

**Conclusions:** This first FcRn drug trial for FMS is expected to deliver information about the potential suitability of this novel treatment approach and the mechanistic importance of proalgic IgG autoantibodies.

#### Article history:

Received 23 February 2024

Received in revised form 3 April 2024

Accepted 6 April 2024

Available online 12 June 2024

#### References

- Andreas G, Clive G, Ulku C, Emerson K, Nisha V, Serena S, Katalin S, Alexandra J, Azar B, Louisa B, Carlos Morado U, Angelika S, Janette T, Diana K, Eva K, Stuart B, Camilla IS, David AA. Passive transfer of fibromyalgia pain from patients to mice. *bioRxiv* 2019:713495. doi: <https://doi.org/10.1101/713495>.
- Blitshteyn S. Autoimmune markers and autoimmune disorders in patients with postural tachycardia syndrome (POTS). *Lupus* 2015;24:1364–9.
- Caro XJ, Winter EF. Evidence of abnormal epidermal nerve fiber density in fibromyalgia: clinical and immunologic implications. *Arthritis Rheumatol* 2014;66:1945–54.
- Caro XJ, Winter EF, Dumas AJ. A subset of fibromyalgia patients have findings suggestive of chronic inflammatory demyelinating polyneuropathy and appear to respond to IVIg. *Rheumatology* 2008;47:208–11.
- Caro XJ, Galbraith RG, Winter EF. Evidence of peripheral large nerve involvement in fibromyalgia: a retrospective review of EMG and nerve conduction findings in 55 FM subjects. *Eur J Rheumatol* 2018;5:104–10.
- Caro XJ. Immunofluorescent detection of igit at the dermal-epidermal junction in patients with apparent primary fibrositis syndrome. *Arthritis Rheum* 1984;27:1174–9.
- Coutinho E, Jacobson L, Shock A, Smith B, Vernon A, Vincent A. Inhibition of maternal-to-fetal transfer of IgG antibodies by FcRn blockade in a mouse model of arthrogryposis multiplex congenita. *Neuro Immunol Neuroinflamm* 2021;8:e1011.
- Cuhadar U, Gentry C, Vastani N, Sensi S, Bevan S, Goebel A, Andersson D. Autoantibodies produce pain in complex regional pain syndrome by sensitizing nociceptors. *PAIN* 2019;160:2855–65.
- European Medicines Agency. EMA/175398/2019—disabling and potentially permanent side effects lead to suspension or restrictions of quinolone and fluoroquinolone antibiotics. Vol. 2023. European Medicines Agency, 2019. Available at: <https://www.ema.europa.eu/en/news/disabling-potentially-permanent-side-effects-lead-suspension-or-restrictions-quinolone-fluoroquinolone-antibiotics>. Accessed May 16, 2024.
- Evdokimov D, Frank J, Klitsch A, Unterecker S, Warrings B, Serra J, Papagianni A, Saffer N, Meyer Zu Altenschildesche C, Kampik D, Malik RA, Sommer C, Uceyler N. Reduction of skin innervation is associated with a severe fibromyalgia phenotype. *Ann Neurol* 2019;86:504–16.
- Freitag H, Szklarski M, Lorenz S, Sotzny F, Bauer S, Philippe A, Kedor C, Grabowski P, Lange T, Riemekasten G, Heidecke H, Scheibenbogen C. Autoantibodies to vasoregulative G-protein-coupled receptors correlate with symptom severity, autonomic dysfunction and disability in myalgic encephalomyelitis/chronic fatigue syndrome. *J Clin Med* 2021;10:3675.
- GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet* 2018;392:1789–858.
- Gibbons CH, Bonyhay I, Benson A, Wang N, Freeman R. Structural and functional small fiber abnormalities in the neuropathic postural tachycardia syndrome. *PLoS One* 2013;8:e84716.
- Gibbons CH, Rajan S, Senechal K, Hendry E, McCaillister B, Levine TD. A double-blind placebo-controlled pilot study of immunoglobulin for small fiber neuropathy associated with TS-HDS and FGFR-3 autoantibodies. *Muscle Nerve* 2023;67:363–70.
- Goebel AS, Schedel R, Frosch M, Roewer N, Sprotte G. IgA Seropositivität für Chlamydia pneumoniae, aber nicht Chlamydia trachomatis, ist assoziiert mit Komplexem Regionalem Schmerzsyndrom (CRPS). *Der Schmerz* 2000;14:70–1.
- Goebel A, Stock M, Deacon R, Sprotte G, Vincent A. Intravenous immunoglobulin response and evidence for pathogenic antibodies in a case of complex regional pain syndrome 1. *Ann Neurol* 2005;57:463–4.
- Goebel A, Jones S, Oomman S, Callaghan T, Sprotte G. Treatment of long-standing complex regional pain syndrome with therapeutic plasma exchange: a preliminary case series of patients treated in 2008–2014. *Pain Med* 2014;15:2163–4.
- Goebel A, Krock E, Gentry C, Israel MR, Jurczak A, Urbina CM, Sandor K, Vastani N, Maurer M, Cuhadar U, Sensi S, Nomura Y, Menezes J, Baharpoor A, Brieskorn L, Sandström A, Tour J, Kadetoff D, Haglund L, Kosek E, Bevan S, Svensson CI, Andersson DA. Passive transfer of fibromyalgia symptoms from patients to mice. *J Clin Invest* 2021;131:e144201.
- Goebel A, Andersson D, Shoenfeld Y. The biology of symptom-based disorders - time to act. *Autoimmun Rev* 2023;22:103218.
- Guo T-Z, Shi X, Li W-W, Wei T, Clark JD, Kingery WS. Passive transfer autoimmunity in a mouse model of complex regional pain syndrome. *PAIN* 2017;158:2410–21.
- Guo T-Z, Shi X, Li W-W, Wei T, Sahbaie P, Clark JD, Kingery WS. Pronociceptive autoantibodies in the spinal cord mediate nociceptive sensitization, loss of function, and spontaneous pain in the lumbar disk puncture model of chronic back pain. *PAIN* 2023;164:421–34.
- Hall J, Bourne KM, Vernino S, Hamrefors V, Kharraziha I, Nilsson J, Sheldon RS, Fedorowski A, Raj SR. Detection of G Protein-Coupled receptor autoantibodies in postural orthostatic tachycardia syndrome using standard methodology. *Circulation* 2022;146:613–22.
- Helyes Z, Tékus V, Szentes N, Pohóczky K, Botz B, Kiss T, Kemény Á, Kőryei Z, Tóth K, Lénárt N, Ábrahám H, Pinteaux E, Francis S, Sensi S, Dénes Á, Goebel A. Transfer of complex regional pain syndrome to mice via human autoantibodies is mediated by interleukin-1-induced mechanisms. *Proc Natl Acad Sci U S A* 2019;116:13067–76.
- Husain M, Chalder T. Medically unexplained symptoms: assessment and management. *Clin Med (Lond)* 2021;21:13–8.
- Iodice V, Kimpinski K, Vernino S, Sandroni P, Fealey RD, Low PA. Efficacy of immunotherapy in seropositive and seronegative putative autoimmune autonomic ganglionopathy. *Neurology* 2009;72:2002–8.
- Kharraziha I, Axelsson J, Ricci F, Di Martino G, Persson M, Sutton R, Fedorowski A, Hamrefors V. Serum activity against G protein-coupled receptors and severity of orthostatic symptoms in postural orthostatic tachycardia syndrome. *J Am Heart Assoc* 2020;9:e015989.
- Krock E, Morado-Urbina CE, Menezes J, Hunt MA, Sandström A, Kadetoff D, Tour J, Verma V, Kultima K, Haglund L, Meloto CB, Diatchenko L, Kosek E, Svensson CI. Fibromyalgia patients with high levels of anti-satellite glia cell IgG antibodies present with more severe symptoms. *bioRxiv* 2022. doi: <https://doi.org/10.1101/2022.07.06.498940>
- Kwan AC, Ebinger JE, Wei J, Le CN, Off JR, Zabner R, Teodorescu D, Botting PG, Navarrete J, Ouyang D, Driver M, Claggett B, Weber BN, Chen P-S, Cheng S. Apparent risks of postural orthostatic tachycardia syndrome diagnoses after COVID-19 vaccination and SARS-cov-2 infection. *Nat Cardiovasc Res* 2022;1:1187–94.

- [29] Levine TD, Kafaia J, Zeidman LA, Saperstein DS, Massaquoi R, Bland RJ, Pestronk A. Cryptogenic small-fiber neuropathies: serum autoantibody binding to trisulfated heparan disaccharide and fibroblast growth factor receptor-3. *Muscle Nerve* 2020;61:512–5.
- [30] Loebel M, Grabowski P, Heidecke H, Bauer S, Hanitsch LG, Wittke K, Meisel C, Reinke P, Volk H-D, Fluge Ø, Mella O, Scheibenbogen C. Antibodies to  $\beta$  adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome. *Brain Behav Immun* 2016;52:32–9.
- [31] Lund H, Hunt M, Kurtovic Z, Sandor K, Fereydouni N, Julien A, Göritz C, Han J, Zhu K, Harris R, Lampa J, Haglund L, Yaksh T, Svensson C. A network of CD163+ macrophages monitors enhanced permeability at the blood-dorsal root ganglion barrier. *bioRxiv* 2023. doi: <https://doi.org/10.1101/2023.03.27.534318>
- [32] Marks K, Vincent A, Coutinho E. Maternal-autoantibody-related (MAR) autism: identifying neuronal antigens and approaching prospects for intervention. *J Clin Med* 2020;9:2564.
- [33] Mazón-Cabrera R, Vandormael P, Somers V. Antigenic targets of patient and maternal autoantibodies in autism spectrum disorder. *Front Immunol* 2019;10:1474.
- [34] McGrath JJ, Saha S, Al-Hamzawi A, Alonso J, Bromet EJ, Bruffaerts R, Caldas-de-Almeida JM, Chiu WT, de Jonge P, Fayyad J, Florescu S, Gureje O, Haro JM, Hu C, Kovess-Masfety V, Lepine JP, Lim CC, Mora ME, Navarro-Mateu F, Ochoa S, Sampson N, Scott K, Viana MC, Kessler RC. Psychotic experiences in the general population: a cross-national analysis based on 31,261 respondents from 18 countries. *JAMA Psychiatry* 2015;72:697–705.
- [35] Nalbandian A, Sehgal K, Gupta A, Madhavan MV, McGroder C, Stevens JS, Cook JR, Nordvig AS, Shalev D, Sehwat TS, Ahluwalia N, Bikdeli B, Dietz D, Der-Nigoghossian C, Liyanage-Don N, Rosner GF, Bernstein EJ, Mohan S, Beckley AA, Seres DS, Choueiri TK, Uriel N, Ausiello JC, Accili D, Freedberg DE, Baldwin M, Schwartz A, Brodie D, Garcia CK, Elkind MSV, Connors JM, Bilezikian JP, Landry DW, Wan EY. Post-acute COVID-19 syndrome. *Nat Med* 2021;27:601–15.
- [36] Oaklander AL, Herzog ZD, Downs HM, Klein MM. Objective evidence that small-fiber polyneuropathy underlies some illnesses currently labeled as fibromyalgia. *PAIN* 2013;154:2310–6.
- [37] Rose NR, Bona C. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 1993;14:426–30.
- [38] Schmack K, Bosc M, Ott T, Sturgill JF, Kepecs A. Striatal dopamine mediates hallucination-like perception in mice. *Science* 2021;372:eabf4740.
- [39] Scott KM, Saha S, Lim CCW, Aguilar-Gaxiola S, Al-Hamzawi A, Alonso J, Benjet C, Bromet EJ, Bruffaerts R, Caldas-de-Almeida JM, de Girolamo G, de Jonge P, Degenhardt L, Florescu S, Gureje O, Haro JM, Hu C, Karam EG, Kovess-Masfety V, Lee S, Lepine JP, Mneimneh Z, Navarro-Mateu F, Piazza M, Posada-Villa J, Sampson NA, Stagnaro JC, Kessler RC, McGrath JJ. Psychotic experiences and general medical conditions: a cross-national analysis based on 28 002 respondents from 16 countries in the WHO World Mental Health Surveys. *Psychol Med* 2018;48:2730–9.
- [40] Sunami Y, Sugaya K, Miyakoshi N, Iwazaki O, Takahashi K. Association of autoantibodies to muscarinic acetylcholine receptors with gastrointestinal symptoms and disease severity in patients with postural orthostatic tachycardia syndrome. *Immunologic Res* 2022;70:197–207.
- [41] Thabit MN, Abdelmomen M, Aboelfadl E, Hadad S. Enhanced sensory conduction in primary fibromyalgia: a case-control pilot study. *Egypt J Neurol Psychiatry Neurosurg* 2021;57:131.
- [42] Toyka KV, Brachman DB, Pestronk A, Kao I. Myasthenia gravis: passive transfer from man to mouse. *Science* 1975;190:397–9.
- [43] Trevino JA, Novak P. TS-HDS and FGFR3 antibodies in small fiber neuropathy and Dysautonomia. *Muscle Nerve* 2021;64:70–6.
- [44] Üçeyler N, Zeller D, Kahn A-K, Kewenig S, Kittel-Schneider S, Schmid A, Casanova-Molla J, Reiners K, Sommer C. Small fibre pathology in patients with fibromyalgia syndrome. *Brain* 2013;136:1857–67.
- [45] Verma V, Drury GL, Parisien M, Özdağ Acarlı AN, Al-Aubodah TA, Nijnik A, Wen X, Tugarinov N, Verner M, Klares R III, Linton A, Krock E, Morado Urbina CE, Winsvold B, Fritsche LG, Fors EA, Piccirillo C, Khouatorsky A, Svensson CI, Fitzcharles MA, Ingelmo PM, Bernard NF, Dupuy FP, Üçeyler N, Sommer C, King IL, Meloto CB, Diatchenko L, HUNT-All in Pain. Unbiased immune profiling reveals a natural killer cell-peripheral nerve axis in fibromyalgia. *PAIN* 2022;163:e821–36.
- [46] Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. *N Engl J Med* 2000;343:847–55.
- [47] Vernino S, Ermilov LG, Sha L, Szurszewski JH, Low PA, Lennon VA. Passive transfer of autoimmune autonomic neuropathy to mice. *J Neurosci* 2004;24:7037–42.
- [48] Wang EY, Mao T, Klein J, Dai Y, Huck JD, Jaycox JR, Liu F, Zhou T, Israelow B, Wong P, Coppi A, Lucas C, Silva J, Oh JE, Song E, Perotti ES, Zheng NS, Fischer S, Campbell M, Fournier JB, Wyllie AL, Vogels CBF, Ott IM, Kalinich CC, Petrone ME, Watkins AE, Yale IMPACT Team, Dela Cruz C, Farhadian SF, Schulz WL, Ma S, Grubaugh ND, Ko AI, Iwasaki A, Ring AM. Diverse functional autoantibodies in patients with COVID-19. *Nature* 2021;595:283–8.
- [49] Yavne Y, Amital D, Watad A, Tiosano S, Amital H. A systematic review of precipitating physical and psychological traumatic events in the development of fibromyalgia. *Semin Arthritis Rheum* 2018;48:121–33.
- [50] Yavne Y, Amital D, Watad A, Tiosano S, Amital H. A systematic review of precipitating physical and psychological traumatic events in the development of fibromyalgia. *Semin Arthritis Rheum* 2018;48:121–33.